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**United States Patent** [19]

Rao et al.

[11] **Patent Number:** **5,990,389**[45] **Date of Patent:** **\*Nov. 23, 1999**[54] **HIGH LYSINE DERIVATIVES OF  $\alpha$ -HORDOTHIONIN**[75] **Inventors:** A. Gururaj Rao, Urbandale; Larry Beach, Des Moines, both of Iowa[73] **Assignee:** Pioneer Hi-Bred International, Inc., Des Moines, Iowa[\*] **Notice:** This patent is subject to a terminal disclaimer.[21] **Appl. No.:** 08/838,763[22] **Filed:** Apr. 10, 1997**Related U.S. Application Data**

[63] Continuation of application No. 08/575,654, Dec. 20, 1995, abandoned, which is a continuation of application No. 08/369,975, Jan. 6, 1995, abandoned, which is a continuation of application No. 08/003,885, Jan. 13, 1993, abandoned.

[51] **Int. Cl.<sup>6</sup>** ..... A01H 5/00; C12N 15/29; C12N 15/82; A01G 13/00[52] **U.S. Cl.** ..... 800/301; 800/279; 530/300; 530/372; 536/23.6; 435/410; 435/419; 435/468; 435/320.1[58] **Field of Search** ..... 800/205, 200, 800/250, DIG. 9, DIG. 52, 301, 298, 278, 279, 295; 435/69.1, 70.1, 172.3, 410, 411, 412, 414, 415, 416, 419, 252.3; 47/58; 536/23.6; 530/300, 372; 514/2[56] **References Cited****U.S. PATENT DOCUMENTS**

5,703,049 12/1997 Rao ..... 514/12

**FOREIGN PATENT DOCUMENTS**

0 318 341	5/1989	European Pat. Off.	.....	C12N 15/00
0 502 718	9/1992	European Pat. Off.	.....	C12N 15/29
WO 89/04371	5/1989	WIPO	.....	C12P 21/00
WO 92/14822	9/1992	WIPO	.....	C12N 15/29
WO 93/03160	2/1993	WIPO	.....	C12N 15/82
WO 93/19190	9/1993	WIPO	.....	C12N 15/29
WO 94/10315	5/1994	WIPO	.....	C12N 15/29
WO 94/16078	7/1994	WIPO	.....	C12N 15/29
WO 94/20628	9/1994	WIPO	.....	C12N 15/82

**OTHER PUBLICATIONS**Altenbach S.B. et al., "Manipulation of Methionine-Rich Protein Genes in Plant Seeds", *Trends in Biotechnology*, vol. 8, No. 6, 1990 pp. 156-160.Altenbach S.B. et al., "Accumulation of a Brazil Nut Albumin in Seed of Transgenic Canola Results in Enhanced Levels of Seed Protein Methionine", *Plant Molecular Biology*, 18: 235-245, 1992 pp. 235-245.Anderson J. et al., "A Transgenic Corn Line with Altered Levels of a High-Methionine Storage Protein", *J. Cell Biochem Suppl.*, vol. 16F, p. 225, 1992, abstract.Beach, L.R. et al., "Enhancing the nutritional value of Seed Crops", *Current Top. Plant Physiol.: Biosynthesis & Molecular Regulation of Amino Acids in Plants*, vol. 7, 1992 pp. 229-238.Florack, D.E.A. et al., "Expression of biologically active hordothionin in tobacco. Effects of pre- and pro- sequences at the amino and carboxyl termini of the hordothionin precursor on mature protein expression and sorting", *Plant Molecular Biology* vol. 24 1994, pp. 83-96.Garcia-Olmeda, F. et al., "Trypsin/alpha-amylase inhibitors and thionins from cereals: possible role in crop protection", *Journal of Exp. Botany Supplement*, vol. 42, No. 238, May 1991 p. 4 and abstract p. 1, 5.Karachi H. et al., "Seed Specific Expression of a Bacterial Desensitized Aspartate Kinase Increases the Production of Seed Threonine and Methionine in Transgenic Tobacco", *The Plant Journal*, vol. 3, No. 5, pp. 721-727, 1993.Karachi H. et al., "Lysine synthesis and catabolism are coordinately regulated during tobacco seed development" *PNAS* 91, 1994 pp. 2577-2581, p. 2581 left column, last line right column.Maddox, J. et al., "Cloning of a barley gene alpha-hordothionin, and expression in transgenic tobacco" *J. Cell Biochem. Suppl.* vol. 16F 1992 p. 217 and abstract p. 212.Rao A.G. et al., "Validation of the Structure-function properties of alpha-hordothionin and derivatives through protein modeling"—see abstract; *Protein Engineering: Supplement, Advances in Gene Technology Protein Engineering and Beyond*, Miami Winter symposium, Jan. 17-22, 1993 vol. 6, 1993.Rao A.G. et al., "Structure-Function Validation of High Lysine Analogs of  $\alpha$ -Hordothionin Designed by Protein Modeling", *Protein Engineering*, vol. 7, No. 12, pp. 1485-1493, Dec. 1994.Florack et al. Synthetic hordothionin genes as tools for bacterial disease resistance breeding. In: *Agricultural Biotechnology in Focus in the Netherlands*, Dekkers et al. eds. Pudoc, The Netherlands, pp. 39-48, 1990.Krebbbers et al. Expression of modified Seed storage proteins in transgenic plants. In: *Transgenic Plants*, Hiatt, ed. Marcel Dekker, Inc., New York, pp. 37-60, 1992.Ponz et al. Cloning and nucleotide sequence of a cDNA encoding the precursor of the barley toxin -hordothionin. *Eur. J. Biochem.* vol. 156, pp. 131-135, 1986.

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**ABSTRACT**Derivatives of  $\alpha$ -hordothionin made by position-specific substitution with lysine residues provide lysine enrichment while retaining the antifungal activity of the parent compound.**21 Claims, 2 Drawing Sheets**

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